background grain counts were scored and subtracted from the nuclear count to yield net grains/nucleus (NG): 1) the maximum count of the two most heavily labelled cytoplasmic areas adjacent to the nucleus, 2) the cytoplasmic area left of the nucleus, 3) the average of the cytoplasmic areas left and right of the nucleus, and 4) the average of two cytoplasmic areas selected by random coordinates on a transparent grid centered over the nuclear image on a TV monitor. Known genotoxins tested using scoring method 1 included dimethylnitrosamine (DMN), diethylnitrosamine (DEN), methylmethanesulfonate (MMS), ethylmethanesulfonate (EMS), 2-acetylaminofluorene (2-AAF), 4-aminobiphenyl (4-AB), benzidine and benzo(a)pyrene (BaP). DMN, DEN, MMS, EMS, 2-AAF and BaP showed strongly positive dose responses (i.e. >=10 NG) comparable to published results from other labs. 4-AB and benzidine were weakly genotoxic (0-5 NG). Selected doses of DMN, DEN, MMS and EMS were rescored with methods 2, 3 and 4, resulting in a marked increase in NG compared to method 1, primarily due to a decrease in the cytoplasmic count. The mean cytoplasmic counts were similar for methods 2, 3 and 4. Further analysis is underway to determine the effect of scoring methods on NG frequency distributions and sensitivity of the assay.

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ANTIMUTAGENIC FACTORS IN COOKED PORK. M. E. Harkins, V. F. Garry, and E. G. Schanus, Environmental Pathology Laboratory, University of Minnesota, Minneapolis, and Washington State University, Pullman.

Mutagenic, carcinogenic, antimutagenic, and anticarcinogenic factors have been found in a wide variety of foods. We have employed the Ames assay to screen for mutagenic and antimutagenic compounds in extracts of cooked pork. Methylene chloride extracts of cooked pork contain material/s which inhibit or suppress the mutagenic activity of direct and indirect mutagens. The test mutagens used in these studies are methyl-nitro-nitrosoguanidine (MNNG), 2-nitrofluorine (2-NF), 2-aminoanthracene (2-AA), and sodium azide (NaN_3) which differ in their chemical mode of mutation induction. Suppression is greatest with MNNG, followed by 2-AA, NaN_3, and 2-NF. MNNG is a monofunctional alkylating agent favoring binding at the O^6 site of guanine, 2-AA is both a monofunctional alkylating agent and intercalates with DNA, only 2-NF is a DNA cross-linking agent. Current studies indicate that isolation and purification of the antimutagenic factor/s is possible using silica gel chromatography. Elutions of the extract with benzene, pyridine, ethanol, and water were tested in the Ames assay and some fractions were found to contain antimutagen activity. Further characterization by normal-phase silica gel HPLC is now underway. Mass spectroscopy and NMR spectroscopy will be used to identify those fractions with antimutagenic properties.

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PROTECTION AFFORDED BY CARNOSINE AND BY ERGOTHIONEINE AGAINST BACTERIOPHAGE P22 INACTIVATION BY γ-IRRADIATION. Philip E. Hartman and Martin J. Citardi, Dept. of Biology, The Johns Hopkins University, Baltimore, MD 21218.

Freifelder (1966, Radiat. Res. Suppl. 6:80) found a striking protection by L-histidine against lethal damage to T7 bacteriophage exposed to X-rays, and the Beattys (1965 Genetics 53:47) found histidine to reduce radiation damage to tradescantia chromosomes under anoxic conditions. High levels of free L-histidine are not found in nature, but the dipeptide carnosine (β-alanyl-L-histidine = CAR) is present in millimolar amounts in striated muscle sarcoplasm of vertebrates. To test the efficiency of CAR as a radioprotector, we exposed Salmonella P22 phage suspended in 0.3 M NaCl to γ-rays from a ^{137}Cs source (Isomedix Gammator B; delivery rate of 279 R/min). At 0.125 and 1 mM CAR protected against lethal damage with a dose-reduction of greater than 8-fold. Dupin et al (1985 Bull. Exp. Biol. Med. 98:1071) report that CAR inhibits accumulation of lipid peroxides in mitochondrial suspensions and in fragments of sarcoplastic reticulum. CAR may be a very important defense against radical damage in vertebrate skeletal muscle. Ergothioneine (2-thiol-L-histidine betaine = ET) is present in mM concentrations in some fungi, for example in Neurospora conidia, and is assimilated and highly conserved in mammals. At a level of 1 mM ET afforded almost complete protection against γ-ray inactivation of P22 phage. We suggest that molecules prevalent at particular sites in nature may serve as protective agents critical in combating macromolecular damage due to free radicals formed during metabolism and oxidative processes. (Supported in part by grant ES03217).